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STUDIES OF THE DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN BRAZIL*

I. POSTEPIDEMIC SURVEY OF MAGÉ, RIO DE JANEIRO, BY COMPLEMENT-FIXATION AND MONKEY-PROTECTION TESTS

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Before the successful maintenance of the yellow fever virus in the laboratory and the development of the protection test with *Macacus rhesus* monkeys, which facilitates the postconvalescent diagnosis of yellow fever,[1] individual suspected cases of this disease could be positively diagnosed only after examination of tissues removed at autopsy. Conclusions regarding the distribution of the disease, therefore, depended on knowledge of the case-fatality rate and the factors which might cause such fatality rate to vary. But experience has shown that, in both epidemic and nonepidemic periods, even fatal cases of yellow fever often pass unnoted and that during epidemics deaths due to other causes are frequently attributed to yellow fever. The difficulties encountered in the diagnosis of nonfatal cases are such that in the absence of epidemic conditions clinicians are unwilling to declare even moderately severe cases. The apparent case-fatality rate varies, therefore, through errors in the clinical diagnosis of both fatal and nonfatal cases.

Furthermore, although the fatality of yellow fever is known to vary

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[1] Stokes, A.; Bauer, J. H.; and Hudson, N. P.: Amer. Jour. Trop. Med., 1928, 8: 103–164 (March).

widely, the factors determining such variation have never been demonstrated. Carter[2] cites yellow fever mortality rates ranging from 4 to 25 per cent in extensive outbreaks, and up to 85 and even 98 per cent in certain "closed-group" epidemics. Variations in the virulence or dosage of the yellow fever virus might help to explain variations in the severity of individual cases or differences between one epidemic and another, but they cannot clarify variations in attack rate and severity in different groups living under identical conditions.

Practically all authors are agreed that in outbreaks of yellow fever in endemic areas the highest attack and fatality rates are to be found among foreigners,[3] especially those who have recently arrived. The disease would appear, from the descriptions given, to exemplify, *par excellence*, Cobbett's[4] general statement: "Peoples are more resistant to the attack of the infectious diseases of their own countries and districts than to that of foreign diseases with which neither they nor their ancestors have been accustomed to come in contact."

The relative freedom of natives and long-time residents in endemic areas from the ravages of yellow fever has been generally attributed to a specific immunity acquired through mild attacks in early childhood or to a partial immunity built up from repeated hypothetical subinfective doses of the virus. In regions having a large negro population native immunity has also been attributed to racial factors. But until recently no method for testing the distribution of immunity was available, and acquired immunity without diagnosed attack could not be distinguished from natural resistance to infection or from absence of exposure.

Although it has long been recognized that recent arrivals from areas in which yellow fever is nonendemic form the group most highly susceptible to the disease, the equally important observation that yellow fever in native populations of endemic areas may be a very elusive disease, even among white adults, has not been sufficiently emphasized. In a place where the disease is permanently endemic it has been al-

[2] Carter, H. R.: In Byam & Archibald's "The Practice of Medicine in the Tropics," 1921–23, 2: 1228–1253. London (Frowde), 3 vols.

[3] The term foreigners, as used in this article, refers to individuals born in areas not subject to endemic or repeatedly epidemic yellow fever.

[4] Cobbett, L.: Tubercle, 1925, 6: 577–590 (Sept.).

most hopeless to expect diagnosis in natives of the place. The epidemiologist engaged in tracing the distribution of yellow fever has therefore been forced largely to supplement with hypotheses the meager scientific information secured from such fatal cases as were followed by autopsy. How faulty such hypotheses, based for the most part on negative findings, may be in endemic areas with little movement of foreigners, is strikingly revealed by the reappearance of yellow fever in the department of Santander, Colombia, in 1929 after an apparent absence from the country for five years and with no evidence pointing to recent reintroduction of the disease from without.[5] Past experience in Brazil has also repeatedly demonstrated that the absence of declared suspected cases of yellow fever from an area does not necessarily mean that the disease itself is absent. Periods of many months without confirmed cases in all Brazil have frequently occurred, only to be followed by more or less extensive outbreaks in the absence, apparently, of any reintroduction from outside sources.

Announcement of the infection of *M. rhesus* monkeys with yellow fever virus (March, 1928) and publication of details of the protection test occurred just as authorities in Brazil were beginning to discover isolated cases of yellow fever, which presaged its wide, known distribution in the country in 1928, 1929, 1930 and 1931. From April, 1927, to March, 1928, no cases of the disease had been confirmed in all Brazil.[6] Early in March, 1928, however, a positive diagnosis was made at autopsy in Estancia, Sergipe (figure 1). In April a fatal case occurred at Timbaúba, Pernambuco. And on May 16 an autopsy was performed in Rio de Janeiro on a soldier dead from the disease. No connection was ever established between any two of these three widely separated cases. Further cases are not known to have occurred in either Estancia or Timbaúba during many succeeding weeks of observation when no control measures were being applied. In Rio de Janeiro, epidemiological studies[7] failed to reveal the original case

[5] Peña Chavarría, A.; Serpa, R.; and Bevier, G.: Jour. Prev. Med., 1930, 4: 417–457 (Nov.).

[6] Confirmation during this period was possible only by autopsy, as other laboratory methods were not yet available.

[7] Barreto, J.: Archivos de Hygiene, 1929, 3: 93–193 (No. 1, May).

or even the original focus of the disease in the federal district. The facts collected demonstrated the existence of at least four distinct foci in Rio de Janeiro in the first half of May (Villa Militar, São Christovão, Saúde and Catumby), but no relationship was ever established between any two of these foci.

FIG. 1

It is impossible to state how long yellow fever had been present in the federal district of Rio de Janeiro before being diagnosed in May, 1928, although a superficial study of general and specific death rates indicates that the disease was probably not an important cause of death in Rio de Janeiro in January, February and March, but may

have been so as early as the first week in April. The impossibility of digging out the past ramifications of the disease in Rio was explained on the basis of the supposed occurrence of a large proportion of mild cases at the beginning of epidemics. After the recognition of the disease, great anxiety was felt for the safety of the city because of the large nonimmune element in the population of the federal district, composed of foreigners, Brazilians from nonendemic regions, and natives of the city born since the end of Oswaldo Cruz's memorable campaign in 1908. Later events justified this anxiety regarding foreigners and Brazilians from nonendemic regions, but, contrary to expectation, there was not an appreciable number of cases among natives of Rio under 20 years of age. Of 125 officially recognized cases in 1928, ninety-seven, or 77.6 ±2.5 per cent, occurred in foreigners, although according to the latest census (1920) only 20.8 per cent of the population are foreigners. Furthermore, 18 of 28 cases (64.3 ±6.2 per cent) occurring in Brazilians were in persons who had resided in Rio de Janeiro less than five years. This failure of Rio de Janeiro to produce in two decades a highly susceptible native population was at first attributed to the fact that the disease predominated in sections of the city, Saúde and Catumby, largely filled with foreigners. While it is true that, with the spread of the disease later to other parts of the city, a significant increase was noted in the percentage of cases occurring among Brazilians (table 1), foreigners continued to suffer disproportionately to their numbers, and some observers suggested that yellow fever may have been more or less constantly present, previous to 1928, in mild or at least undiagnosed form, thus maintaining a high degree of immunity in the native population. But this hypothesis is not reasonable, because Rio de Janeiro has had a constantly shifting foreign population and has, moreover, long been the Mecca of the Portuguese immigrant; this highly susceptible material would undoubtedly have given rise to explosive outbreaks in the past had the virus been present in sufficient quantity to immunize the local population.

There was continued difficulty throughout 1928 and 1929 in tracing the source of individual infections, although the fatality of diagnosed cases was very high, 59.6 ±1.2 per cent. For many of the cases which occurred the only apparent explanation was the assumption

that a considerable number of undiagnosed yellow fever infections were present even during the height of the epidemic when the medical profession and the public were both "yellow-fever-minded."

TABLE 1*

Distribution of cases of yellow fever treated in the Hospital São Sebastião, Rio de Janeiro, between 1892 and 1899, and cases diagnosed in Rio de Janeiro in 1928, in 1929 and in 1928 and 1929

	HOSPITAL, 1892–99	RIO, 1928	RIO, 1929	RIO, 1928–29
Total cases	14,546	125	613	738
Males	13,042	100	461	561
Females	1,502	25	152	177
Per cent males	89.7 ±0.2	80.0 ±2.4	75.2 ±1.2	76.0 ±1.1
Brazilians	701	28	203	231
Foreigners	13,845	97	335	432
Unclassified			75	75
Per cent foreigners classified	95.2 ±0.1	77.6 ±2.5	62.3 ±1.4	65.2 ±1.2
Whites	14,309	120	485	605
Mulattoes	107		43	
Blacks	126	5	4	53
Yellow			1	
Unclassified			80	80
Per cent whites classified	98.4 ±0.1	96.0 ±1.2	91.0 ±0.8	91.9 ±0.7
In Rio less than 3 years		92	268	360
In Rio more than 3 years		33	65	98
Unclassified			280	280
Per cent classified in Rio less than 3 years		73.6 ±2.6	80.5 ±1.5	78.6 ±1.3
Age 20 to 40 years	9,083	83	308	391
Other ages	5,463	42	220	262
Unclassified			85	85
Per cent age 20–40 classified	62.4 ±0.2	66.4 ±2.8	58.3 ±1.4	59.4 ±1.3

* Data from A. da S. Mello (Brasil Medico, 1928, 42: 1079–87, Sept.), J. Barreto (Archivos de Hygiene, 1929, 3: 93–193, No. 1, May), C. Fraga (Brasil Medico, 1930, 44: 1081 and 1113, Sept. 27 and Oct. 4), and S. Vianna (in C. Fraga's "A Febre Amarella no Brasil," 1930, pp. 221–229).

The distribution of cases of yellow fever occurring during the Rio de Janeiro epidemic of 1928 and 1929 is compared in table 1 with the

distribution of cases treated in the isolation hospital in Rio from 1892 to 1899. These figures are probably not strictly comparable, as the 1892–99 figures refer only to hospitalized cases and would therefore probably tend to include a larger percentage of cases among foreigners than among Brazilians, as well as a larger percentage of cases among men than among women. Even so, an analysis of table 1[8] shows that the absence of yellow fever from the city for two decades was not entirely without effect. Significant differences are found in the percentages of cases among Brazilians, among females, and among whites in the two periods studied. *No appreciable difference is found however in the percentage of total cases occurring between the ages of 20 and 40 years.*

Mello[9] calls attention to the fact that the observed distribution of diagnosed cases in the Rio de Janeiro epidemic of 1928 corresponded closely to the observed distribution of cases during the period when yellow fever was endemic in the city. He hypothecates a *nonspecific immunity to infection* in natives and old-timers which he attributes, following Gordon,[10] to long-continued exposure to the bites of *A. aegypti*, with the production of a local tissue immunity in the skin. Mello points out the failure of the investigations of the health authorities to reveal evidence of any significant number of mild abortive cases in Rio to explain the selectivity of the disease and its unexpected distribution, and refuses to accept specific acquired immunity due to invisible infection as the only explanation of the observed facts. While admitting that mild undiagnosable infections with the virus of yellow fever do occur, he insists on the validity of the ancient belief in an immunity of acclimatization, which can be progressively lost, by either natives or old-time residents in endemic areas, through continued residence in cold nonendemic climates. He adduces this belief as an added argument for an unstable nonspecific immunity in persons long resident in endemic regions, who have never suffered from attacks of the disease.

Barreto[7] notes the hypothesis that lowered basal metabolism observed in residents of tropical countries may contribute to greater

[8] In considering table 1 the predominating influence of the classification "foreigners" on the other classifications presented must not be forgotten.

[9] Mello, A. da S.: Brasil Medico, 1928, 42: 1079–1087 (Sept.).

[10] Gordon, R. M.: Ann. Trop. Med. & Parasitol., 1922, 16: 229–234 (Oct.).

resistance to infection, but concludes (p. 93) that "the relative scarcity of cases in children depends really rather on the difficulties of the clinical diagnosis than on the paucity of patients."

In considering the attack and fatality rates of yellow fever in the recent outbreak in Rio de Janeiro it is interesting to know that Torres Homem and other early writers on the 1849–50 epidemic in that city, which occurred after an apparent absence of yellow fever from Brazil during many decades, reported that foreigners were far more susceptible to the disease than natives. A like observation, made at Bahia under similar conditions the same year, occurs in the report of the President of the Province of Bahia for 1850:[11] "The number of natives attacked between October, 1849, and the end of June, 1850, exceeded one hundred thousand, but among them the disease was much milder than among the foreigners. The mortality among the former was 3 to 4 per cent, but among the latter it was more than 30 per cent."

One of us (FLS) observed at close range the Rio de Janeiro yellow fever outbreak of 1928–29 and early came to the conclusion that information of value might be secured if it were possible to carry out protection tests on a group of native-born residents living in known infected zones of the city. As reasonable certainty existed that yellow fever had not been endemic in Rio de Janeiro on a large scale since 1908, natives less than 20 years of age found to have a specific immunity would most probably have a recently acquired immunity. Plans were made for a series of protection tests, and in January, 1929, work was begun with Dr. Hugo Muench in Braz de Pinna, a suburb of Rio de Janeiro. The press of other services, however, made it necessary to discontinue this study. Before the project could be undertaken anew, yellow fever was reported in the nearby town of Magé, in the State of Rio de Janeiro, where, during February, March, and April, a small epidemic of 22 cases with 13 deaths occurred.[12] Magé offered a much more concrete problem than did Braz de Pinna and was chosen as the site of the present study in the belief that the results might be

[11] Pereira, A. P.: "Conferencia sobre peste bubonica e febre amarella," 1910, Bahia (Associacão Commercial da Bahia), 91 pp.

[12] Lintz, A., and Parreiras, D.: "Notas e estudos epidemiologicos sobre a febre amarella (1928–1930)." Nictheroy, 1930.

applied with profit to the interpretation of the observed distribution of yellow fever following the reintroduction of the virus into Rio de Janeiro, an old endemic center, after an apparent absence of twenty years.

After the work in Magé had been begun (June, 1929), the first report of promising results with the complement-fixation test for yellow fever was published.[13] In view of the low cost of this test, it was decided to examine a much larger number of persons in Magé than would have been possible with the more expensive protection test, and provision was made for a series of control tests with sera from a noninfected center. Piracicaba, São Paulo (figure 1), lying outside the endemic yellow fever zone, was selected for the collection of sera (1930) for the control series. Later (1931) an opportunity occurred to secure a control series of sera from another known postepidemic focus of yellow fever at Santo Aleixo, a small town near Magé.

The data secured from these investigations are the basis of the present report, which gives: (1) comparative results of complement-fixation tests in Magé and Santo Aleixo, both lying in a previously endemic area and both the seats of recent epidemics, and of similar tests in Piracicaba, São Paulo, believed to have been free of yellow fever for many years; (2) comparative results of complement-fixation tests in Magé, four, sixteen and twenty-two months after a known epidemic of yellow fever; (3) comparative results of complement-fixation and *M. rhesus*-protection tests in Magé; (4) an analysis of yellow fever immunity distribution in the population of Magé in 1929, as indicated by complement-fixation and *M. rhesus*-protection tests.

YELLOW FEVER IN MAGÉ AND SANTO ALEIXO

Magé, the county seat of Magé county, is situated at the junction of two railways, by one of which Rio de Janeiro may be reached in about an hour and a half (figure 1). Contact with Rio is also maintained by small freight and fishing sailboats. The life of Magé depends largely on the operation of the local cotton mill, which is reported to have some five hundred people in its employ. The population, roughly estimated at 2500 for 1929, is, on the whole, a remark-

[13] Frobisher, M., Jr.: Proc. Soc. Exper. Biol. & Med., 1929, 26: 846-848 (June).

TABLE 2

*Results of unofficial census of Magé, January, 1925**

RACE OR COLOR	AGE (YEARS)							TOTAL	PER CENT
	Under 1	1 to 4	5 to 9	10 to 14	15 to 19	20 to 49	50 and over		
Indian	0	0	0	2	1	1	0	4	0.2
Negro	12	36	48	61	39	138	32	366	16.2
Mulatto	20	65	80	76	58	234	52	585	25.9
White	36	153	178	154	151	507	121	1,300	57.7
Total	68	254	306	293	249	880	205	2,255	
Per cent	3.0	11.2	13.6	13.0	11.0	39.0	9.1		

* From Boyd, M. F., et al.: Amer. Jour. Hyg., Monograph Series 5, 1926 (May).

ably stable one, with distribution by race and age as shown in table 2. That part of the population studied for the present report showed the following distribution by age groups, corresponding closely to that of table 2:

AGE (YEARS)	NUMBER	PER CENT
Under 4	209	13.8
5 to 9	189	12.4
10 to 14	212	14.0
15 to 19	178	11.7
20 to 49	594	39.0
50 and over	139	9.1
Total	1,521	100.0

Santo Aleixo and the contiguous village of Andorinhas, forming one nucleus of population, comprise about five hundred houses lying largely between the two cotton mills which justify their existence. This center, which will be referred to as Santo Aleixo in this report, lies within the county of Magé, some eighteen kilometers inland from the town of Magé, with which it is connected by a small steam rail line operated for the convenience of the cotton mills.

The history of yellow fever in Magé and Santo Aleixo is believed to have depended in the past on the history of the disease in Rio de Janeiro. These towns, like Rio, are not believed to have harbored yellow fever between 1908 and 1928.

Although Magé is close to, and in daily contact with, Rio de Janeiro, there were no rumors of yellow fever in the former town before December, 1928, and no proof of its presence there until February 13, 1929, when the first positive autopsy findings were made. Control measures, including antilarval work, medical inspection, isolation of the sick and fumigation, were instituted on March 21 by the Yellow Fever

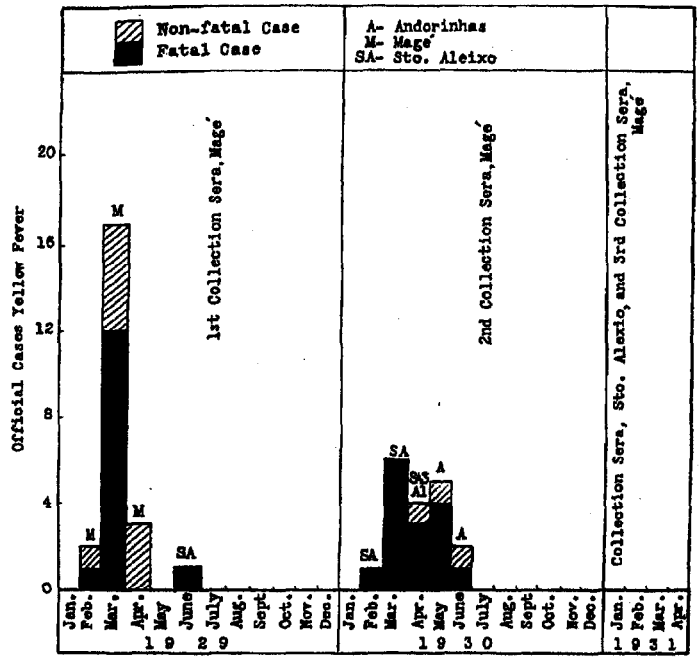

FIG. 2. DISTRIBUTION, BY MONTHS, OF OFFICIALLY RECOGNIZED CASES OF YELLOW FEVER IN MAGÉ AND SANTO ALEIXO, WITH REFERENCE TO COLLECTION OF BLOOD SAMPLES FOR TESTS

Service of the State of Rio de Janeiro. The last known case in Magé occurred April 8, 1929. The distribution of officially recognized cases in Magé and Santo Aleixo is shown in figures 2 and 3. The official duration of the epidemic was nine weeks. The last cases occurred eighteen days after control measures were begun, and the fatality of cases recognized before the health authorities arrived was much higher than that of later cases. Both these facts may be interpreted to mean that many more cases probably occurred in Magé than were diagnosed.

The two physicians practicing in Magé differed regarding the extent of the yellow fever outbreak. One believed that many cases had occurred in the town during January, February and March, with comparatively few deaths, while the other, although admitting that yellow fever had been present in the town and that he had seen two cases with suspicious symptoms, believed that most of the fever cases seen by him had been due to malaria.

The monthly number of medical calls and consultations among families of employees of the cotton mill made by one physician, be-

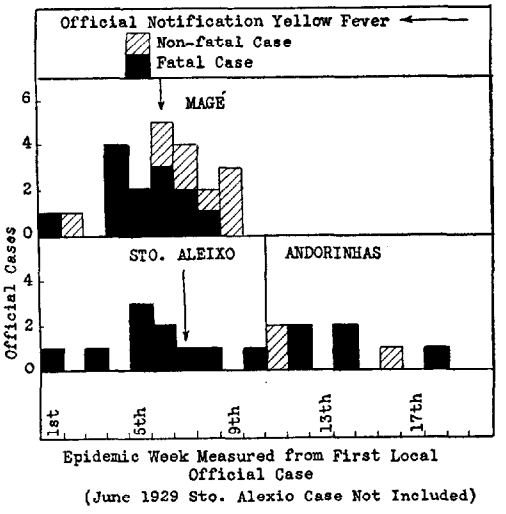

FIG. 3. DISTRIBUTION, BY EPIDEMIC WEEKS, OF OFFICIALLY RECOGNIZED CASES OF YELLOW FEVER IN MAGÉ AND SANTO ALEIXO

ginning with October, 1928, were: 185; 193; 234 (December); 224 (January—yellow fever probably present in December and January); 295; 544; 303 (yellow fever present in these months of February, March, April); 162 (May—yellow fever probably absent); 131 (June —yellow fever surely absent, present study begun).

While the great increase in the number of calls noted in February, March, and April covers the period during which yellow fever is known to have been present in Magé, it also corresponds to the season of greatest prevalence of malaria. It is fortunate, from the standpoint of the present study, that Magé is one of the four places which had

been chosen in 1922 by Boyd[14] for comparative studies on the epidemiology of malaria in the State of Rio de Janeiro. Boyd's conclusions after three years' study were (p. 111): "We have the villages of Sant' Anna, Porto das Caixas, and Itamby with a very high incidence of endemic malaria, the fluctuations of which among the fixed population do not vary widely from year to year, while, on the other hand, *we have in Magé an area of low endemicity, subject from time to time to epidemic exacerbations.*"

A moderate epidemic of malaria had occurred in Magé in 1922 before Boyd's first blood index had been taken and control measures begun. Boyd reports blood indices for the town in the months of July and August 1922–24 as follows:

YEAR	EXAMINED	POSITIVE	INDEX
1922	1,009	375	37.2
1923	1,352	139	10.3
1924	1,208	59	4.9

Had Magé suffered a widespread epidemic of malaria in February, March and April, 1929, the blood index based on slides examined in June of the same year should have been high. Such was not the case; only 32 of 932 blood films were positive. In spite of the fact that the 1929 index was taken one month nearer to the epidemic season than were Boyd's indices, this index, 3.4 $\pm$ 0.4, is the lowest recorded for Magé. The distribution of residence of the persons whose blood was found positive for malaria in June, 1929, shows no similarity to the distribution of cases of suspected yellow fever or of persons whose blood sera gave a positive complement-fixation reaction. Furthermore, none of the persons whose blood was positive for malaria are among those listed as having had cases of suspected yellow fever. The authors believe that malaria may be eliminated as a cause of the large number of medical calls registered by the factory physician.

It is unfortunate, so far as the present study is concerned, that deaths from a considerable district outside the town of Magé are registered with those of the town itself. The most recent official census (1920) gives the total population of the registry district as 6216,

[14] Boyd, M. F., et al.: Amer. Jour. Hyg., Monograph Series 5, 1926 (May).

which is more than double that of the town itself. However, even though rates cannot be ascertained, the raw data from the registry district are sufficiently interesting to be presented here, although no definite conclusions from them are justified.

As will be seen from table 3 and figure 4, more deaths were registered in March, 1929, than in any other month during the ten-year period, 1920–29. The number of deaths registered during each month of 1929 was in excess of the nine-year average for that month; and in each

TABLE 3

Registered deaths in Magé registration district by months, 1920 to 1929

	1920	1921	1922	1923	1924	1925	1926	1927	1928	TOTAL	AVERAGE DEATHS, 1920–1928	PROBABLE ERROR OF DISTRIBUTION OF DEATHS, 1920–1928	1929	RATIO DIFFERENCE IN 1929 DEATHS FROM AVERAGE TO THE PROBABLE ERROR OF 1920–1928 DISTRIBUTION
January	12	6	5	7	14	3	8	9	8	72	8.0	±2.2	9	0.46
February	11	14	8	9	7	4	4	8	12	77	8.6	±2.2	12	1.6
March	15	11	9	12	10	9	7	10	11	94	10.4	±1.4	22	8.1
April	14	8	4	6	10	5	6	10	8	71	7.9	±2.0	12	2.08
May	7	8	4	14	8	7	7	10	5	70	7.8	±1.8	10	1.20
June	5	6	5	8	6	9	7	4	13	63	7.0	±1.7	8	0.57
July	2	19	19	6	4	8	7	6	9	80	8.9	±3.9	14	1.30
August	3	6	8	7	1	5	3	7	11	51	5.7	±1.9	12	3.27
September	10	3	9	8	5	2	1	6	7	51	5.7	±2.0	11	2.65
October	2	6	10	6	6	4	5	8	8	55	6.1	±1.5	8	1.25
November	5	4	12	8	3	5	8	8	7	60	6.7	±1.7	14	4.21
December	5	7	4	8	5	7	3	10	11	60	6.7	±1.7	15	4.88
Annual total	91	98	97	99	79	68	66	96	110	804	89.3	±10.7	147	5.40

of five months (March, August, September, November and December) the number of deaths registered exceeded the previous maximum for that month. In March, August, November and December, the 1929 registered deaths exceeded the upper limits of the nine-year averages plus three times the probable errors of the distributions from 1920 to 1928 for these months. Deaths for March showed an increase over the nine-year average of more than eight times the probable error of the March distribution, and total deaths for the year 1929 showed an increase over the average of almost six times the probable

error of the annual distribution. The drop to normal in June was followed by an increase during the rest of the year, with the result that the mortality in the district continued much above normal even

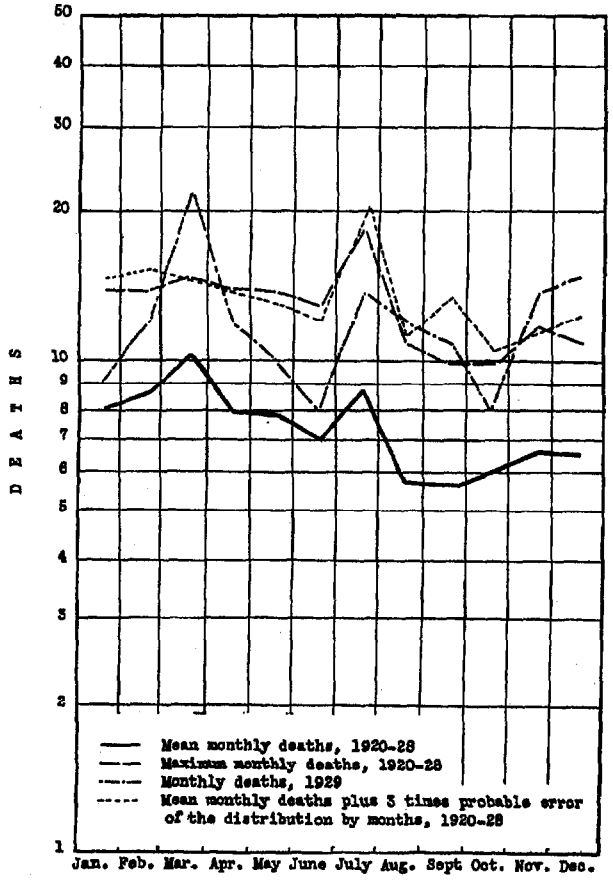

FIG. 4. DEATHS IN MAGÉ REGISTRATION AREA BY MONTHS (SEMILOGARITHMIC SCALE)

after the inauguration of mosquito control and the disappearance of yellow fever from the town itself. That this persistent high death rate in the district may have been associated with the persistence of yellow fever in the rural areas is suggested by the occurrence in Febru-

ary, March, April, May and June, 1930, of cases of yellow fever in Santo Aleixo, Andorinhas and nearby rural areas within the county of Magé[12] without evidence of reintroduction of the disease.

It is interesting to note that the peak of the known cases of yellow fever and the peak of sick calls, discussed above, fall in the same month as the maximum general mortality.

Registered deaths by age groups for the period 1920 to 1929, inclusive, are shown in table 4 and figure 5. Increases over the average for the previous nine-year period are noted for all age groups in 1929, such increases being more than three times the probable errors of the

TABLE 4

Registered deaths in Magé registration district by age groups, 1920 to 1929

AGE	1920	1921	1922	1923	1924	1925	1926	1927	1928	TOTAL, 1920–1928	AVERAGE DEATHS, 1920–1928	PROBABLE ERROR OF DISTRIBUTION OF DEATHS, 1920–1928	1929	RATIO DEFERENCE IN 1929 DEATHS FROM AVERAGE TO THE PROBABLE ERROR OF 1920–1928 DISTRIBUTION
Under	17	24	24	35	24	23	30	41	35	253	28.1	±4.8	30	0.39
1 to 4	40	21	16	22	19	15	16	17	29	195	21.7	±5.2	39	3.36
5 to 9	8	7	6	3	6	1	3	3	8	45	5.0	±1.6	7	1.23
10 to 14	2	5	2	1	0	1	0	2	4	17	1.9	±1.1	7	4.75
15 to 19	3	5	6	1	1	0	3	1	4	24	2.7	±1.3	6	2.54
20 to 49	9	17	27	23	17	19	9	17	16	154	17.1	±3.7	28	2.95
50 and over	12	19	16	14	12	9	5	15	14	116	12.9	±2.6	30	6.60
Total.....	91	98	97	99	79	68	66	96	110	804	89.3	±10.7	147	5.40

distributions for ages 1 to 4, 10 to 14, 20 to 49, and over 50. For all ages above 10 years, the total deaths in 1929 were equal to, or greater than, the maxima for these groups during the previous nine-year period.

Santo Aleixo, in spite of its proximity to and dependence on Magé, was not known to harbor the virus of yellow fever until the first week in April, 1930, one year after the occurrence of the last registered case in Magé. Santo Aleixo, having no resident physician, depends on lay observers for certification of cause of death in those persons not seen by the cotton-mill company's physician on his scheduled visits to the community. The Yellow Fever Service for the State of Rio de

Janeiro, alarmed at receiving from Santo Aleixo certificates in which the cause of death was given by laymen as "yellow-typhoid" and "hemorrhagic-typhus," ordered autopsy on all persons dying in the

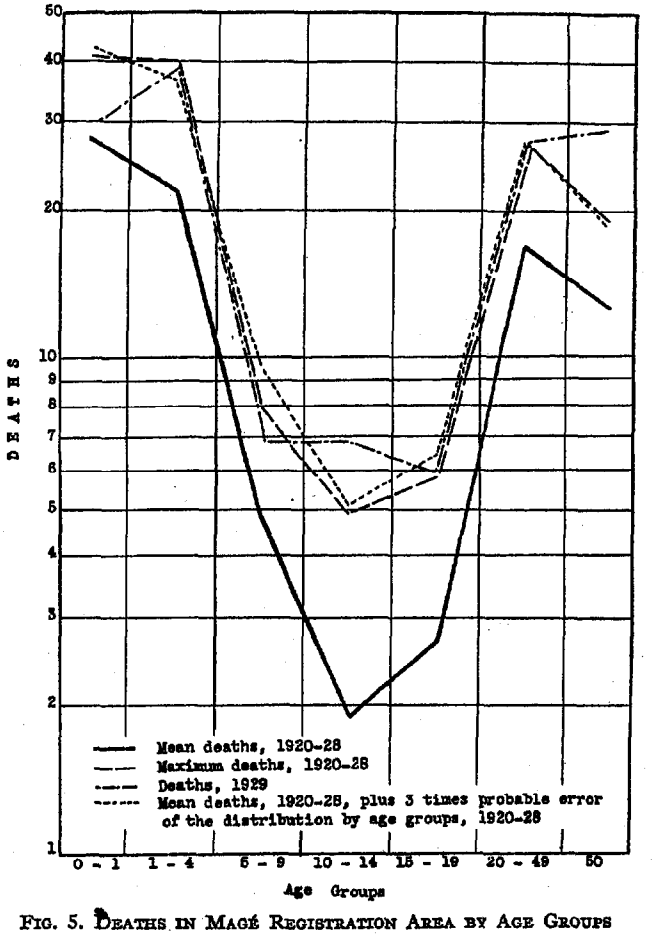

FIG. 5. DEATHS IN MAGÉ REGISTRATION AREA BY AGE GROUPS

community. On April 5 it received notification of a death occurring on March 29, which autopsy had shown to be due to yellow fever.[12] Retrospective investigation made at this time caused a diagnosis of yellow fever to be made for seven previous fatalities occurring without

medical attention between June 1, 1929, and March 25, 1930, with the following causes of death certified by laymen: malaria (2 cases), hemorrhagic-typhus (2 cases), pulmonary congestion, gastro-enteritis, and yellow-typhoid. After the discovery of the disease in Santo Aleixo in April, eleven additional cases were observed in this center—three in Santo Aleixo proper and eight in the contiguous Andorinhas. The distribution of the eleven officially recognized cases in Santo Aleixo and the eight in Andorinhas is shown in figures 2 and 3. If Santo Aleixo and Andorinhas are considered as a unit, the official epidemic period is eighteen weeks, but if considered separately the period for Santo Aleixo is ten weeks and that for Andorinhas eight weeks.

COLLECTION OF MATERIAL

Magé. In June, 1929, a house-to-house canvass was made in the central part of Magé, and epidemiological data were collected regarding 1521 people living in 348 houses. During this survey 36 suspected cases of yellow fever were added to the official list of 22. Some of these were relatively mild cases and would not have suggested yellow fever under other circumstances. The distribution of the officially recognized cases and the additional suspected cases is roughly the same. At this same time (June, 1929) thick blood smears were taken from 932 residents of the study area to obtain the malaria index,[15] and in July and August, 1929, blood samples of 10 to 30 cc. for complement-fixation tests were secured from 676 persons, 2 to 81 years of age, living in the area. In July, 1930, second blood samples were secured from 88 persons who had furnished samples in 1929; and in January, 1931, additional samples were taken from 34 persons who had furnished specimens on one or both of the previous occasions (tables 6 and 7).

Santo Aleixo. In January, 1931, blood samples of 10 to 30 cc. were secured from 70 persons living in Santo Aleixo and Andorinhas, most of whom were less than 20 years of age.

Piracicaba, State of São Paulo. As a control on the results of complement-fixation tests in Magé, 120 blood samples were secured in September, 1930, from persons of all ages living in Piracicaba, a busy town of some 25,000 residents in the interior of the State of São Paulo.

[15] Results discussed above (page 353).

Piracicaba is situated about 140 miles from Santos at an altitude of more than 500 meters above sea level, and yellow fever is not known to have been present there during the past thirty-five years.

METHODS

All samples for tests were taken in vacuum venules, which are indispensable where many specimens are to be collected in homes and shops. The sera, after separation, were drawn off aseptically, placed in sterile tubes or ampules, and sent to the laboratory for as early examination as possible.

The complement-fixation test was made, using an antigen prepared from the liver of monkeys infected with yellow fever. The technique of this test has been adequately described elsewhere.[15,16]

The protection test consisted in injecting 3 or 4 cc. of serum intraperitoneally into *M. rhesus* monkeys, one serum to each monkey. Within five minutes each animal received an infective dose of citrated blood of an animal in the early febrile stage of yellow fever, usually infected with the Asibi strain of virus. A control animal which received no serum was given a similar dose of infective blood at the same time as each group of test animals. Rectal temperatures of all animals were taken twice daily. Autopsies were performed on animals that died, and the gross and microscopic pathological findings were recorded. Interpretation of tests on monkeys surviving febrile attacks was difficult, but after weighing evidence for and against protection, partial protection, and nonspecific reactions, most of these tests could be recorded as positive or negative.

COMPLEMENT-FIXATION TESTS

Comparative results in Magé and Santo Aleixo and in Piracicaba. The results of complement-fixation tests in Magé in July, 1929, in Santo Aleixo in January, 1931, and in Piracicaba in September, 1930, are presented in table 5. The percentages positive in Magé and Santo Aleixo are equal, whereas the difference in percentages positive in Magé County and in Piracicaba is sufficiently large in proportion to its probable error to make it highly significant.

Results four, sixteen and twenty-two months after the Magé epidemic.

[16] Frobisher, M., Jr.: Jour. Prev. Med., 1931, 5: 65–78 (Jan.).

The results of complement-fixation tests on the sera of 54 persons in Magé four and sixteen months, and four and twenty-two months, after an epidemic of yellow fever are given in tables 6 and 7. A large number of sera were tested, but only those giving entirely satisfactory readings in both the tests are included. A statistical analysis of these tables shows that, although there is considerable tendency for early positive complement-fixation tests to become negative, there is, nevertheless, a high degree of correlation between the results of successive tests.

Results of parallel complement-fixation and protection tests. The results of protection tests with sera from Magé (1929) giving clearly

TABLE 5

Summary of complement-fixation tests, Magé, Santo Aleixo, and Piracicaba

LOCALITY	BLOOD DRAWN	SPECIMENS TESTED	SATISFACTORY REACTIONS*	POSITIVE TESTS	PER CENT POSITIVE
Magé	July, 1929	370	250†	105	42.0 ±2.1
Santo Aleixo	January, 1931	60	50	21	42.0 ±4.7
Total, Magé County		430	300	126	42.0 ±1.9
Piracicaba	September, 1930	120	101	3	3.0 ±1.1
Difference in per cent positive, Magé County and Piracicaba					39.0 ±2.2

* That is, not anticomplementary.

† These sera had been stored in ampules for some time, which may account for the fact that only 68 per cent of the reactions were satisfactory.

positive or clearly negative complement-fixation readings are shown in table 8. It will be seen that positive complement-fixation results were generally confirmed by positive protection tests, whereas an appreciable number of sera giving negative complement-fixation reactions protected *M. rhesus* against yellow fever virus. In spite of the tendency toward an excess of positive protection tests over positive complement-fixation tests, statistical analysis of table 8 shows a high degree of correlation between complement-fixation and protection test results.

In table 9 are presented comparative results of complement-fixation and protection tests with the sera of 18 persons in Magé giving a

history of an illness in 1929 suspected to have been yellow fever. Of these, 3 were anticomplementary and could not be read, 12 were positive, one was negative, and two were doubtful. Protection tests on *M. rhesus* with the sera of 9 of these 18 persons confirmed 7 positive

TABLE 6

Results of successive complement-fixation tests with sera of the same persons taken at Magé four and sixteen months after an epidemic of yellow fever*

	1930		TOTAL	PER CENT CHANGE FROM 1929 RESULTS
	POSITIVE	NEGATIVE		
1929:				
Positive	11	15	26	57.7 ±6.5
Negative	1	27	28	3.6 ±2.4
Total	12	42	54	25.9 ±7.8

* No cases of yellow fever are known to have occurred in the town of Magé after April 8, 1929, although cases were confirmed for the county in June, 1929, and from February to June, 1930. Data from Lintz and Parreiras: "Notas e estudos epidemiologicos sobre a febre amarella (1928–1930)." Nictheroy, 1930.

TABLE 7

Comparative results of complement-fixation tests with sera of same persons taken at Magé four and twenty-two months after an epidemic of yellow fever (1929 and 1931)

	1931		TOTAL	PER CENT CHANGE FROM 1929 RESULTS
	POSITIVE	NEGATIVE		
1929:				
Positive	10*	3‡	13	23.1 ±8.6
Negative	2†	9‖	11	18.2 ±8.7
Total	12	12	24	4.2 ±9.8

* Two of six of these tested in 1930 were negative.
† One of these tested in 1930 was negative.
‡ Two of these tested in 1930 were both negative.
‖ Three of these tested in 1930 were all negative.

and one negative complement-fixation test and indicated that one doubtful complement-fixation test should have been negative. In other words, nine persons with recent history of suspected yellow fever gave closely corresponding results by the two test methods. Of

TABLE 8

Results of protection tests with sera giving readable complement-fixation reactions, Magé

	COMPLEMENT-FIXATION TEST			PER CENT CONFIRMED BY COMPLEMENT-FIXATION TEST
	Positive	Negative	Total	
Protection tests:				
Positive	28	14	42	66.7 ±4.9
Negative	3	31	34	91.2 ±3.3
Total	31	45	76	
Per cent confirmed by protection test	90.3 ±3.6	68.9 ±4.7		

TABLE 9

Results of complement-fixation and protection tests in 18 persons affected in Magé in 1929 with an illness suspected to have been yellow fever

CASE NUMBER	AGE	NATIVITY	DATE OF ATTACK	COMPLEMENT-FIXATION*		PROTECTION 1929
				1929	1930	
15	52	Portugal	March	AC	not done	not done
17	26	Italy	March	+	not done	+
194	24	Syria	March	+	not done	+
195	21	Brazil	March	+	not done	+
266	32	Portugal	March	+	+	not done
267	29	Portugal	March	+	+	not done
268	6	Portugal	March	+	+	not done
655	48	Portugal	March	+	not done	+
657	18	Brazil	March	+	not done	+
658	16	Brazil	March	−	not done	−
1181	35	Syria	April	AC	not done	not done
1187	12	Brazil	February	AC	not done	not done
1188	15	Brazil	April	?	not done	−
1235	39	Brazil	March	+	not done	+
1238†	13	Brazil	April	+	not done†	not done
1239	8	Brazil	March	+	not done	+
1241	8	Brazil	March	−?	not done	not done
1248	38	Brazil	March	+	not done	not done

* AC = Anticomplementary.

† Complement-fixation test January, 1931, positive.

the persons whose sera gave positive complement-fixation reactions in 1929, three were reëxamined in 1930 and one in 1931, and all again gave positive reactions.

YELLOW FEVER IMMUNITY DISTRIBUTION IN MAGÉ IN 1929 AS INDICATED BY COMPLEMENT-FIXATION AND M. RHESUS-PROTECTION TESTS

The distribution of positive and negative complement-fixation reactions, according to the part of Magé in which the donor of the blood sample resided, does not, to casual inspection, show a marked difference. However, if the results of tests are grouped with respect to occurrence of officially recognized cases of yellow fever, there appears to be a definite relationship between *known infection* of a group of contiguous houses and a high percentage of positive complement-fixation reactions, especially in the more closely built-up central part of the town.

TABLE 10

Results of complement-fixation tests in Magé, according to place of residence

RESIDENCE	EXAMINED	COMPLEMENT-FIXATION		PER CENT POSITIVE
		Positive	Negative	
In central blocks with cases 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 19, 20, 21, and 22*	118	66	52	56.0 ±3.4
Outside above listed blocks	132	39	93	29.6 ±2.7
Total	250	105	145	42.0 ±2.1
In house with case	18	10	8	56 ±7.9
In neighboring house	43	20	23	47 ±5.1
Others	189	75	114	40 ±2.4
Total	250	105	145	40 ±2.1

* Cases numbered in order of date of onset.

In those central blocks in which occurred fifteen known cases (table 10) a significantly higher percentage of complement-fixation tests was positive than in other parts of Magé, although the earliest known cases and possibly the longest period of infection occurred in these other areas. The differences in percentages positive in the groups "in house with case," "in neighboring house," and "others" (table 10) are very suggestive, but an analysis of the relationship of these differences to their probable errors indicates that such differences cannot be considered statistically significant.

The classification of results according to the color of the donor of the blood specimen (table 11) reveals that there is a significant difference

in percentages positive in whites and blacks. There is no racial segregation in Magé, although there may be some tendency for blacks to live away from the center of population. To minimize this possible effect of residence, all persons tested were classified as to color and to proximity to known cases of yellow fever, and whites still seem to give a higher percentage of positive reactions than do either mulattoes or negroes, although the numbers in each group are too small to give significance to the differences noted. Protection tests on *M. rhesus* with sera from Magé, although few in number, seemingly confirm the

TABLE 11

Results of complement-fixation tests in Magé, according to color and residence

COLOR	RESIDENCE								PER CENT POSITIVE*			
	In house with suspect		In neighboring house		Others		Total		In house with suspect	In neighboring house	Others	Total
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative				
White.....	7	6	10	8	44	52	61	66	54 ±9.3	56 ±7.9	46 ±3.4	48 ±2.9
Mulatto.....	3	1	9	11	21	38	33	50	75 ±14.6	45 ± 7.5	36 ±4.2	40 ±3.6
Black.....	0	1	1	4	10	24	11	29	0	20 ±12.0	29 ±5.2	28 ±5.0
Total.....	10	8	20	23	75	114	105	145	56 ±7.9	47 ±5.1	40 ±2.4	42 ±2.1

* No statistically significant differences are shown above when probable errors of differences are taken into consideration, except between total blacks and total whites, with difference of 20 ±5.8 per cent.

results of the complement-fixation test, protection being afforded by sera from twenty (60.6 ±5.7 per cent) of thirty-three whites, fourteen (56 ±6.7 per cent) of twenty-five mulattoes, and only eight (44 ±7.9 per cent) of eighteen blacks tested. This apparent difference in racial susceptibility is in accord with clinical observation, but an inspection of probable errors shows that these results are not statistically significant.

Although the official list of yellow fever cases occurring in Magé in 1929 shows only two cases in foreigners, at least five others occurred in foreign families whose children born in Brazil are listed as Brazilian. Likewise, among the 36 additional suspected cases discovered in the

present study, 14 occurred in foreigners or in children of foreign parents. This number is out of proportion to the number of foreigners in Magé and emphasizes the greater susceptibility of those of foreign blood to clinical yellow fever.

TABLE 12

Results of complement-fixation and protection tests in males and females, Magé

	COMPLEMENT-FIXATION			PROTECTION		
	Positive	Negative	Per cent positive	Positive	Negative	Per cent positive
Males	49	80	38 ±2.9	21	17	55.3 ±5.4
Females	56	65	46 ±3.3	21	17	55.3 ±5.4
Total	105	145	42 ±2.1	42	34	55.3 ±3.8

TABLE 13

Results of complement-fixation and protection tests by age groups, Magé

AGE (YEARS)	COMPLEMENT-FIXATION				PROTECTION TEST			
	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive
Under 4	2	3	5	40 ±19.1	0	0	0	
5 to 9	11	12	23	48 ±10.4	3	2	5	60 ±19.1
10 to 14	13	13	26	50 ±6.5	5	2	7	71 ±11.5
15 to 19	13	30	43	30 ±4.7	5	14	19	26 ±6.8
20 to 29	27	32	59	46 ±4.5	11	5	16	68.7 ±7.8
30 to 39	22	22	44	50 ±6.1	11	4	15	73.5 ±7.7
40 to 49	11	14	25	44 ±6.7	4	3	7	57.1 ±14.9
50 and over	6	19	25	24 ±5.7	3	4	7	42.9 ±14.9
Total	105	145	250	42 ±2.1	42	34	76	55.3 ±3.8
Under 19	39	58	97	40 ±3.4	13	18	31	41.9 ±6.0
20 and over	66	87	153	43 ±2.7	29	16	45	64.5 ±4.8
Total	105	145	250	42 ±2.1	42	34	76	55.3 ±3.8

Males and females show the same percentage of positive protection tests (table 12). The difference between percentages in positive complement-fixation tests is less than two times its probable error and hence not highly significant.

Classification in accordance with whether the donor was above or

below 20 years of age (table 13), a grouping based on the probable date of the last previous outbreak of yellow fever in Magé, shows that the results of complement-fixation tests are practically uniform in the two groups, whereas the results of protection tests jump from 41.9 ±6.0 per cent positive in the younger group, to 64.5 ±4.8 per cent positive in the group over 20 years of age. This difference (22.6 ±7.7 per cent) is almost three times its probable error. The difference between the percentage positive by complement-fixation tests and that positive by protection tests in the group over 20 years of age (21.5 ±5.5 per cent) is significant. When only complement-fixation tests are considered, a significant difference (20 ±6.1 per cent) is found between the percentage positive in the age group under 49 years and that positive in the group 50 years and over.

The results of complement-fixation tests by age groups for Santo Aleixo are as follows:

AGE (YEARS)	EXAMINED	POSITIVE
Under 4	0	0
5 to 9	11	3
10 to 14	22	8
15 to 19	13	7
20 to 29	2	1
30 to 39	1	1
40 to 49	1	1
50 and over		
Total	50	21
Per cent positive		42.0 ±4.7

DISCUSSION

In addition to possible technical errors in performing the complement-fixation and protection tests, there appear to be other factors influencing the results of these tests. As the complement-fixing and protection bodies, although formed in response to the same stimulus, are apparently not identical, factors causing an increase or decrease in one element may not affect the other. Errors in either protection or complement-fixation tests, then, should affect the degree of correlation found between results of the two tests.

M. rhesus-protection tests may be falsely positive because of normally resistant animals. These are estimated at "not more than 6 per cent" by Beeuwkes, Bauer and Mahaffy,[17] who have undoubtedly had the largest experience with the test. The same authors, who feel the necessity of using duplicate animals for each test, report that in 274 duplicate tests there were 38 instances (13.9 ±1.4 per cent) in which only one of two test animals died. Evidence of the difficulty of interpreting protection tests was also encountered in our tests. Twenty-five of 76 protection tests (33 ±3.6 per cent) were not entirely clear cut and were interpreted on the basis of previous experience with the test. Falsely negative *M. rhesus*-protection tests do occur, and consistent results are not always obtained with serum from the same individual.

Patient N. C. D., who had yellow fever in April, 1929 (Burke and Davis),[18] has failed to react clinically to repeated feedings of numerous batches of infected mosquitoes over a period of eighteen months. Serum of N. C. D. has given the following results in monkey protection tests during 1929: May and July, prevented death but not fever; September, no protection; October, full protection; November, severe fever and recovery; December, full protection.

Beeuwkes, Bauer and Mahaffy[17] also cite a case in which the serum from a person known to have had yellow fever failed to protect one of a pair of *M. rhesus* monkeys, after having repeatedly protected *M. rhesus* in previous tests. It is not clear whether these inconsistencies are due (1) to uncertainties of the test itself, because of variation in susceptibility of individual monkeys and of variation in activity of virus, either of which might cause variable results in working with serum of low titer of protection bodies, or (2) to variation in the titer of protection bodies in the serum of the same individual, possibly dependent on opportunity for reinfection.

By analogy with results obtained in monkeys, complement-fixing bodies may be expected to appear in the sera of human yellow fever patients several days after the onset of the disease, or later, and to increase gradually to a maximum, to be followed in many cases by a

[17] Beeuwkes, H.; Bauer, J. H.; and Mahaffy, A. F.: Amer. Jour. Trop. Med., 1930, 10: 305–333 (Sept.).

[18] Burke, A. W., and Davis, N. C.: Amer. Jour. Trop. Med., 1930, 10: 419–426 (Nov.).

decline.[19, 20, 21] It seems probable, however, that a considerable number of persons will maintain a supply of complement-fixing bodies over a long period of time. Hudson[22] has reported complement-fixation tests on the sera of five persons who had contracted yellow fever in the laboratory. One of these persons failed to develop complement-fixing bodies during eight months of observation, while another showed persistence of complement-fixing bodies twenty-one months after infection. No estimate can be made at the present time of the percentage of persons who will fail to develop complement-fixing bodies.

Macacus rhesus monkeys in which the complement-fixing titer of the blood has fallen very low some months after infection have, when reinoculated with the virus of yellow fever, shown an increase of complement-fixing titer to the previous maximum titer or beyond, without exhibiting other signs of reinfection (G. E. Davis,[20] Frobisher).[18] Similar reactions may be expected to occur in human beings. Whether such repeated exposures to the virus are capable of producing a permanently higher titer of complement-fixing bodies in the blood is unknown.

It should be noted that the first three of the above-mentioned factors leading to a disparity between the results of complement-fixation and protection tests (namely, resistant monkeys, delayed appearance or absence of complement-fixing properties, and nonpermanence of complement-fixing properties) all tend to give a greater number of positive protection tests than of positive complement-fixation tests. The effect of possible variations in titer is more difficult to foretell.

In spite of the several factors of variation, the results of the Magé complement-fixation tests show a high degree of correlation with the Magé protection tests (tables 8 and 9), and the results of the Magé postepidemic complement-fixation tests are widely different from those obtained with sera from Piracicaba, where yellow fever is not endemic. The results of postepidemic complement-fixation tests in Magé also correspond closely, as to percentage positive, with the results of similar tests at Santo Aleixo. In Magé, 90 ±3.6 per cent of positive complement-fixation tests and only 69 ±4.6 per cent of negative complement-

[19] Frobisher, M., Jr.: Amer. Jour. Hyg., 1931, 13: 586–613 (March).

[20] Davis, G. E.: Amer. Jour. Hyg., 1931, 13: 79–128 (Jan.).

[21] Monteiro, J. L., and Travassos, J.: Memorias do Instituto Butantan (São Paulo), 1930, 5: 173–191.

[22] Hudson, N. P.: Proc. Soc. Exper. Biol. & Med., 1931, 28: 937–939.

fixation tests were confirmed by protection tests. Analyzing the figures of table 8 from the opposite point of view, it is noted that 67 ±4.9 per cent of positive protection tests and 91 ±3.3 per cent of negative protection tests were confirmed by the results of complement-fixation tests. In other words, 90 ±3.6 per cent of positive complement-fixation tests were confirmed by protection tests and 91 ±3.3 per cent of negative protection tests were confirmed by complement-fixation tests, but only 69 ±4.6 per cent of negative complement-fixation tests were confirmed by protection tests and only 67 ±4.9 per cent of positive protection tests were confirmed by complement-fixation tests. These results are important and indicate that the complement-fixing properties in the blood of convalescents are less constantly produced and less permanent than are the elements responsible for protection. This suggestion is in part borne out by the results of repeated complement-fixation tests on the same individuals, which indicate that there may be a rapid decline of positive reactions in the first years after an epidemic (tables 6 and 7). In Magé the percentages of positive complement-fixation tests in the age groups below and above 20 years were equal, whereas a much higher percentage of protection tests was positive in the age group over 20 years. If the presence of complement-fixing bodies always represents a recent infection, one would be forced to conclude that the older age group, in spite of residence during childhood in an endemic area, had produced as high a percentage of infections as had the younger age group. However, if complement-fixing bodies are in most cases only present for a short period of time and are produced anew in the presence of the virus without production of disease,—which, as has been shown above, is what occurs in *M. rhesus*—an equal percentage of positive complement-fixation results should be obtained in all age groups following reintroduction of the virus after a long absence. The spread between positive complement-fixation tests and positive protection tests in the older age group, then, may represent roughly the number of immunes in Magé who were not subjected to the virus anew in 1929. An attempt was made to calculate the probable percentage of immunity in the age group above 20 years, previous to the most recent introduction of the disease, using the formula:

$$x + r(100 - x) = y$$

in which r is the apparent attack rate of the present epidemic as indicated by protection tests in the age group under 20 years, and by complement-fixation tests in all groups, and y is the final postepidemic percentage of immunity indicated by the protection test for the age group over 20 years. The solution of the equation:

$$x + .4\,(100 - x) = 64.5$$

indicates that approximately 41 per cent of the age group over 20 years were immune before the last outbreak.

G. E. Davis,[20] although reporting excellent results with the complement-fixation test in monkeys, concludes on the basis of a small series of largely negative results that the complement-fixation test can be of no assistance in determining endemic areas of yellow fever. Although there are at present difficulties in the interpretation of individual complement-fixation results in endemic areas, comparison of the results in Magé, Santo Aleixo and Piracicaba (table 5) indicates that the complement-fixation test may have a definite place in outlining endemic, or at least recently epidemic, areas. Results obtained by Monteiro and Travassos[21] support this belief. These authors report thirty-three (49 ±4.2 per cent) positive complement-fixation reactions—twenty (29.8 ±3.8 per cent) strongly positive and an additional thirteen weekly positive—in tests on sera of 67 persons in Bahia, an old endemic center of yellow fever, but no positive reactions in tests on sera of 20 Lithuanian and Japanese immigrants from whom blood samples were secured within 24 hours of arrival in Brazil.

The Piracicaba data, taken in conjunction with the high percentage of positive complement-fixation reactions in Magé, which were confirmed by positive protection tests, indicate that very few falsely positive complement-fixation tests will be read. Results are sufficiently striking to warrant making further comparative studies of protection and complement-fixation tests in areas where the history of yellow fever is known, before discarding the complement-fixation test or assuming that its results are not important in the study of endemic areas.

The percentage of positive protection tests in Magé (55.3 ±3.8) is not statistically different from that (68.0 ±6.3) reported by Beeuwkes, Bauer and Mahaffy[17] from Ife in Nigeria after an epidemic

of yellow fever. Endemic areas may be expected to show a greater divergence between positive complement-fixation and positive protection tests than do areas recently epidemic.

From a study of material herewith presented Magé is believed to have been subject, during the first four months of 1929, to a rather extensive outbreak of yellow fever, characterized by a large number of mild immunizing infections and by very few severe and fatal cases. The results of complement-fixation and protection tests, taken in conjunction with the low malaria index found, are interpreted as indicating that the number of medical calls in January, February, March and April, when yellow fever is known to have been present, was probably due to so-called abortive attacks of yellow fever. The analysis of immunity distribution in Magé, as shown by the complement-fixation test in the present study, failed to reveal statistically significant differences between groups variously classified by age, sex, and race.

Even if the excess of total deaths for the epidemic period in the Magé district be attributed to yellow fever, the evidence still indicates that the fatality rate in Magé was actually very low, although the official fatality rate was 59 $\pm$7.0 (13 deaths in 22 cases). As the strain of virus responsible for the Magé epidemic was most probably imported from Rio de Janeiro, it is interesting to note that the official fatality rate in Rio during the same year was 59 $\pm$1.3. Since virus, climate and previous history of yellow fever are similar for Magé and Rio de Janeiro, may it not be possible that a large number of abortive infections were occurring in the latter city also? The official fatality rate in Santo Aleixo is believed, in the light of known facts and the results of the complement-fixation test, to be greatly exaggerated because of incomplete registry of cases.

Many clinical observers have in the past expressed a belief in the occurrence of mild cases of yellow fever in endemic areas, but the conditions under which these take place have never been established. The tendency of epidemiologists has been to ignore the possibility of their occurrence except in infants and in negroes, and these two groups have largely been held responsible for all that is difficult to explain in the epidemiology of yellow fever in endemic areas.

Practically all observers agree that the foreigner, newly arrived from an area where yellow fever is not endemic, runs the greatest risk of

infection and suffers most severely from the disease. On no other factor believed to influence susceptibility, however, is there unanimity of observation and opinion. Many authors assert that infants and small children are relatively immune to the disease, whereas Hansen[23] reports a high fatality rate in this group.

The negro race[24] is generally believed to suffer less from yellow fever than do other races, and yet Boyce,[25] who observed the Barbados epidemic of 1909, calls attention (p. 238) to the large percentage of cases occurring in blacks, with a high death rate. The same author comments on the outbreak of yellow fever in Freetown, West Africa, in 1884, in which cases of the disease, but no deaths, were reported among the native-born blacks. Boyce attributes the mildness of the disease in blacks in Freetown to previous attacks of the disease, a position untenable in the light of recent laboratory experience with the protection test.

Ramsey[26] cites the Asibi case (Stokes, Bauer and Hudson)[1] as a proof that yellow fever in the black may be a very mild disease, and interprets data from Senegal on the basis of race. However, in Brazil the yellow fever virus has several times been isolated from whites whose attacks of the disease were so mild that a positive diagnosis could not have been made on clinical grounds. The first two reported infections of *M. rhesus* with South American virus (Aragão)[27] were produced with blood from immigrants who had had mild cases of the disease; and the S.R. strain of virus reported upon by N. C. Davis and Burke[28] was obtained from a Spanish woman whose infection was very light. Dr. P. J. Crawford succeeded (October, 1930) in establishing the yellow fever virus from a native white child (Morlin), aged 12 years, who had a three-day fever and headache, without vomiting, jaundice, albuminuria or other symptoms suggestive of yellow fever.

[23] Hansen, H.: Amer. Jour. Trop. Med., 1929, 9: 233–239 (July).

[24] It must be remembered that the so-called "negro race" is composed of many widely variant racial strains which probably are not uniform in resistance to yellow fever. Sawyer and Lloyd (Jour. Exper. Med., 1931, 54: 533–555—Oct.) have demonstrated wide variations in resistance to yellow fever encephalitis in various strains of white mice.

[25] Boyce, Sir R. W.: "Yellow Fever and Its Prevention," 1911, London (J. Murray), 480 pp.

[26] Ramsey, G. H.: Amer. Jour. Hyg., 1931, 13: 129–163 (Jan.).

[27] Aragão, H. de B.: Mem. Inst. Oswaldo Cruz (Rio de Janeiro), Suppl. No. 2, 1928, pp. 23–46 (Oct. 15).

[28] Davis, N. C., and Burke, A. W.: Jour. Exper. Med., 1929, 49: 975–984 (June).

The present study indicates that, during an epidemic in a previously endemic region, a large part of the native population may develop a specific immunity to the virus of yellow fever without presenting the classical symptoms of the disease. This unsuspected immunization occurs in various age groups and is not limited to the black race. No definite indication is given, however, as to the factors which determine the mild or negative symptoms which accompany this immunization.

The epidemiology of yellow fever has had no more devoted observer and student than the late Dr. H. R. Carter[2] who, after presenting widely varying fatality rates for different outbreaks (p. 1244), asks: "*Is the fever milder among the natives of countries in which it is endemic or of frequent occurrence—i.e., is there an hereditary immunity?* I have thought so from what I have seen in purely white communities of the United States on the Gulf of Mexico, but it is nowise proven."

The influence of individual heredity in determining resistance to disease is again being emphasized by the scientific world after several decades during which the causative organism alone had been considered. Jennings[29] goes so far as to state: "It is probable that there is no disease whatever, acute or chronic, infectious or noninfectious, whose occurrence is not influenced by the nature of the individual's genetic constitution."

An hereditary immunity, or rather resistance, to the effects of yellow fever infection, whether due to the direct transmission of protection from parents to children, as suggested by Carter's observations, or to the selective action of the virus with elimination of a large part of the more susceptible genetic combinations from the race, would explain a surprising number of observed facts and would harmonize many of the divergent opinions held by students of the disease. First, it would constitute a most logical explanation for the one point on which all observers are agreed, namely, that yellow fever is most severe among foreigners. Negroes have not in the past moved in appreciable numbers from areas where yellow fever does not occur to zones where it is endemic. The disease in negroes has usually been observed, then, in natives of endemic, or repeatedly epidemic areas and might be

[29] Jennings, H. S.: "The Biological Basis of Human Nature," 1930, p. 149. New York (W. W. Norton & Co.).

expected, on the hypothesis of hereditary resistance, to show a low fatality rate. Assuming an inherited resistance to yellow fever, the Barbados (1909) and the Freetown (1884) experiences cited above can be reconciled on the basis of limited previous outbreaks with little inherited resistance or incomplete elimination of susceptible strains in Barbados, and of extensive previous outbreaks in Freetown.

An inherited resistance might be expected to be most effective in early childhood, and the number of cases observed in infants of newly-arrived foreigners must be very limited; both these factors would help to explain the impression that yellow fever is mild in children. On the other hand, a population highly resistant because of the elimination of the more susceptible gene combinations might be expected, since such elimination can never be complete in a human population, to show occasional typically severe and fatal cases even in the early age groups, inasmuch as, on this hypothesis, resistance is based on genetic constitution and not on gradually diminishing protection bodies inherited from the immediate parents. Such severe cases in infants are known to occur in highly endemic areas where both parents are of native stock and apparently possess long-standing immunity to yellow fever. That the apparent mildness of the disease in children is not entirely due to age is evident from the reports on fatality of yellow fever in children in nonendemic regions. Hansen[23] reports 19 deaths in 23 cases in children under two years of age (a fatality rate of 82.6 $\pm$5.3), and 14 deaths in 27 cases in children between two and five years of age (a fatality rate of 52.0 $\pm$6.5) during an epidemic of yellow fever occurring in Peru after the disease had been absent for twenty-five years.

The observation that the mortality in old people is very high may be due in part to the fact that very few natives of endemic regions live to an old age without having had the disease. Hence the classification of "old age" here will often carry the hidden classification of "foreigner."

Either of the above suggested hereditary mechanisms would admirably explain the fact that when yellow fever was reintroduced into Rio de Janeiro in 1928 and 1929, after a lapse of two decades, there did not occur among the natives of the city a large number of reported cases or of deaths. On a larger scale, the hypothesis of inherited resistance to yellow fever virus and the consequent occurrence of many

subclinical[30] cases in the natives of endemic areas, irrespective of race and age, explains observed facts much better than does the assumption of mild attacks limited to negroes and infants. However, an affirmative answer to Carter's query need not carry the implication that his corollary of an inherited resistance necessarily holds, or that, even if present, such hereditary resistance is the most important factor in the production of mild infections. The hypothesis of hereditary resistance fails to explain the low incidence of clinical yellow fever in foreigners more than five and less than twenty years in Brazil in the recent Rio outbreak. Neither does it explain the belief in the loss of immunity by natives and old-time residents living for some years in nonendemic areas.

More and more epidemiologists are coming to realize the possible importance of nonspecific factors—such as chemical composition of diet, vitamin content of diet, variation in basal metabolic rate, exposure to sunlight, variations in temperature and humidity—in the production of seasonal and group variations in attack and fatality rates of infectious disease, although the mechanism whereby such factors intervene in the infectious process has not been determined. For example, Aycock[31] calls attention to a number of seasonal variations in physiological processes and shows that such seasonal variations conform to the same law as that which determines the occurrence of poliomyelitis. Without attempting to link resistance to the virus of poliomyelitis with any one of these processes, he suggests that there may be some variation in the physiological activity of the body which influences resistance to the disease. This author has coined the word *autarcesis* to distinguish this nonspecific "protective power against disease which exists in the body by reason of a normal or balanced physiological activity," from specific immunity, the result of actual invasion of the body by the disease-producing agent.

Variations in temperature and humidity influence the prevalence of yellow fever by their direct action on the insect host of the virus, but it has also been suggested that climatic factors and exposure to the uninfected insect host over long periods of time may result in a sum total of acclimatization capable of giving the population of certain

[30] Not necessarily without symptoms, but without symptoms recognizable as yellow fever.

[31] Aycock, W. L.: Jour. Prev. Med., 1929, 3: 245–278 (May).

regions an increased relative resistance to the dangerous results of infection with the yellow fever virus.

The possible influence of variations in metabolism and of antecedent dietary habits on the course of an infection which may produce such pronounced destruction of liver tissue as does yellow fever should not be overlooked in view of the known protective action of certain food substances against the liver destruction caused by such agents as chloroform (N. C. Davis)[32] and carbon tetrachloride (N. C. Davis,[33] Minot and Cutler).[34] In this connection it is interesting to note that some of the highest fatality rates reported for yellow fever have been observed in "closed groups," such as companies of soldiers and crews of ships, which, in addition to fulfilling the requirement of "newly-arrived foreigners," had also been subjected to uniform and probably well-below-optimum diets.

SUMMARY AND CONCLUSIONS

1. Complement-fixation tests on postepidemic blood samples taken from 300 persons in Magé and Santo Aleixo and on 101 samples from Piracicaba, lying outside the yellow fever zone, show a highly significant difference between the percentages of positive reactions in the postepidemic samples and in the samples from the nonendemic area. The conclusion is drawn that the complement-fixation test may be useful in field studies of yellow fever, especially in outlining areas recently epidemic.

2. A small number of comparative results of complement-fixation tests on the same individuals in Magé at intervals of four, sixteen and twenty-two months after an epidemic of yellow fever indicate that there is a tendency, especially in the first year after infection, for the titer of the complement-fixing bodies in the blood to decrease.

3. Comparative results of complement-fixation and *M. rhesus*-protection tests with 76 sera indicate that positive complement-fixation tests will generally be confirmed by positive protection tests and that negative protection tests will usually be confirmed by negative complement-fixation tests. However, an appreciable number of

[32] Davis, N. C.: Arch. Int. Med., 1919, 23: 612–635 (May).
[33] Davis, N. C.: Jour. Med. Res., 1924, 44: 601–614 (Sept.).
[34] Minot, A. S., and Cutler, J. T.: Jour. Clin. Investigation, 1928, 6: 369–402 (Dec.).

sera giving protection showed negative results with the complement-fixation technique. These findings indicate that complement-fixing bodies demonstrable by present methods are less constantly produced than are protection bodies, and are less permanent.

4. The combined results of complement-fixation and protection tests given in this report are interpreted as indicating that, at the time of known outbreaks of yellow fever in Magé and Santo Aleixo, relatively large percentages of the local populations were acquiring a specific immunity to the yellow fever virus without apparent attacks of the disease. A statistical analysis of the available data failed to show that such invisible acquisition of active immunity was related in any way to age, sex or race. No data are presented to indicate what factors are responsible for this ability of the population of these two towns, lying in previously endemic areas, to acquire active immunity with low fatality rates.

5. The possibility of low fatality rates with many unnoted infections in natives of endemic or previously endemic yellow fever areas, without regard to age, sex, or color, must be taken into consideration in any future attempt to study the distribution of the yellow fever virus.